Observations of overwintering nymphalid butterflies in underground shelters in SW and W Bohemia (Czech Republic) (Lepidoptera: Nymphalidae: Nymphalini)

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Abstract. The results from ten years of surveys and observations of overwintering nymphalid butterflies in various types of underground shelters in SW-W Bohemia, Czech Republic are presented. During these surveys, three species of nymphalid butterflies were encountered; the most commonly observed species was *Inachis io* (Linneaus), followed by *Aglais urticae* (Linneaus). *Nymphalis polychloros* (Linneaus) was encountered in the ten year period only twice. The typical overwintering sites for *Inachis io* were the ceilings of unheated cellars of buildings, and the entrances of mining tunnels/galleries or natural caves. A special category of underground shelters in the study area was abandoned World War 2 military bunkers. *Inachis io* frequently hibernates in aggregations of several individuals and *Aglais urticae* does so less often. The lowest ambient air temperature measured in these underground shelters was −1.1° C. It is believed that these observations represent the longest continuous record of hibernating nymphalid butterflies in underground (or other) shelters in Czech Republic.

Key words: phenology, ecology, overwintering sites, winter diapause, hibernation, Anglewing butterflies, butterfly conservation, natural resource protection

Introduction

The Anglewing butterflies are a small group of nymphaline butterflies inhabiting the Northern Hemisphere (ca. 35 sp.). It is generally agreed that this group is a natural monophyletic clade, based on several synapomorphic characters including special adaptations that enable the adults to survive the winter in hibernation (hibernal diapause) (see Scott, 1979). Recent phylogenetic studies (Nylin *et al.*, 2001; Wahlberg & Nylin, 2003; Wahlberg *et al.*, 2005) confirmed the monophyletic relationship for the Anglewing butterflies as a group. For the purposes of this paper we recognize the genera *Aglais*, *Inachis*, *Nymphalis*, and *Polygonia*. However, it may turn out that there are only two genera: *Aglais* and *Nymphalis* comprise the Anglewing butterflies. If that is the case, then *Inachis* would become a junior subjective synonym of *Aglais* and likewise *Polygonia* becomes a junior subjective synonym of *Nymphalis*. For the purposes of this paper we recognize the genera *Aglais*, *Inachis*, *Kaniska*, *Nymphalis*, *Polygonia* and *Roddia*. The genus *Kaniska* is not represented in Europe, and the Euro-Asian *Roddia l-album* does not occur recently in Bohemia. There are a few more or less anecdotal notes regarding overwintering nymphalines from Bulgaria (Beshkov & Petrov, 1996), former Czechoslovakia (*cf.* Košel, 1984; Dvořák, 2000, 2002), France (Sarlet, 1982), Germany (Bronner, 1987; Herhaus & Karthaus, 1996), Poland (Kowalski, 1955), Spain (Escola, 1982) and others. This paper reports observations of primarily *Inachis io* (L.) and *Aglais urticae* (L.).

With the onset of fall, typically at the end of August, these butterflies begin to search for suitable overwintering shelters. Depending on the species, the selected sites vary. The most frequently observed

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overwintering shelters include crevices beneath roofing shingles; animal burrows; cavities in hollow trees; rock and branch piles; attics, barns and other outbuildings; unheated cellars; caves; and mining tunnels and galleries. Once in the shelter, the butterfly falls into a stupor as the temperature drops. The freezing point of their cell tissue is lowered by an increased content of glycerols and sugars, which act as an antifreeze (Pullin & Bale, 1989a, b). The freeze tolerance seems to vary between species, as evidenced by the selection of different wintering sites. Using electrical conductivity, K. W. Philip (pers. com.) found that adults of *Nymphalis antiopa* in Fairbanks, Alaska do not freeze until the temperature reaches -30°C. In the spring, the hibernal diapause is broken when a period of cold weather passes.

Most butterflies typically live no longer than thirty days, but some overwintering butterflies can live up to almost a year (Guppy & Shepard, 2001). This remarkable longevity is a direct adaptive product of adult hibernal diapause.

An immobile butterfly is very vulnerable to discovery by a predator (Wiklund & Tullberg, 2004). To minimize the detection by birds, mice and other predators, Anglewing butterflies have evolved cryptic coloration and pattern on the ventral side of their wings (Fig. 1). In normal posture, the hibernating butterfly folds its wings over its back, exposing only the ventral side. This minimizes its silhouette and butterfly blends in with the background (camouflage).

Survey methods

Study area and wintering sites

The overwintering sites visited during the surveys included underground shelters such as caves, mining tunnel and gallery entrances, military bunkers, and unheated cellars of castles, monasteries, and residential houses. A few natural karstic, pseudokarstic and/or fissure caves were also visited. The 10-year observation period started in the winter of 1994–1995 and continued until the winter of 2003–2004. Altogether, 347 locations were surveyed in this period. Some of locations were visited only once; some of them were visited several times during one winter. A total of 2265 visits were made in this period. Table 1 summarizes the wintering sites and localities. The total number of wintering butterflies was recorded during each visit to each site. In one instance, a long mining tunnel was surveyed in five metre intervals for all wintering Lepidoptera. Some results have been previously partly reported by Dvořák (2000, 2002).

Figure 1. *Nymphalis j-album,* Cummington Fairgrounds, Hampshire County, Massachusetts.

Hibernating Butterfly taxa

Most Czech butterflies overwinter as pupae or larvae. Of the 161 species recently reported for Czech Republic by Beneš *et al.* (2002), only the nine species are known to overwinter as adults. These are: *Gonepteryx rhamni* (L.) [Pieridae: Pierinae], *Nymphalis polychloros* (L.), *N. xanthomelas* Esper, *N. l-album* Esper, *N. antiopa* (L.), *Inachis io* (L.), *Aglais urticae* (L.), *Polygonia c-album* (L.) [all Nympahidae: Nymphalinae: Nymphalini]. Of these, *N. l-album* and *N. xanthomelas* do not occur in SW Bohemia. *Polygonia c-album*, which hibernate in central Europe, evidently do not hibernate in underground shelters (Wiklund & Tullberg, 2004). One specimen was found on the wall to the entrance to the cellars of the Přečin castle in SW Bohemia on 31.XII.2006 (L. Dvořák, unpubl. data).

Other allied nymphalid butterflies, such as *Araschnia levana* (L.) hibernate as pupae; and the vanessids, such as migratory *Vanessa cardui* (L.), apparently do not hibernate at all (Pollard *et al.*, 1998); *V. atalanta* (L.), which is also migratory, can occasionally hibernate in central Europe (Tucker, 1991; Hensle, 2000). It is interesting to note that Schappert (2000) observed *Vanessa atalanta* and *V. virginiensis* in mid-March in Ontario, Canada, in addition to overwintered individuals of *Nymphalis antiopa, N. j-album, Aglais milberti.*

Over a period of 10 years, individuals of three species – *Aglais urticae, Inachis io,* and *Nymphalis polychloros* – were observed using various underground shelters as their hibernation sites. Table 1 summarizes the observations.

Statistics

We analysed the occurrence of nymphalids in wintering shelters by general linearized models (GLM) with a Poisson distribution of the dependent variable (number of individuals) separately for each species, using S-Plus 2000 package (Splus, 2000, 1999). To delimit effects of space autocorrelation, we included variables of place and space to all models. The effect of differences due to year was entered into analyses both as a continuous and as a categorical variable.

Results

Nymphalids in different shelters

Three species, *A. urticae*, *Inachis io*, and *N. polychloros*, were found in the underground shelters in the study area. However all three species were found together only in the abandoned military bunkers. These bunkers are typically concrete structures, partially buried in the ground. The species most frequently encountered there was *Inachis io*, which seems to hibernate there very regularly (more than 82% from 45 visited bunkers). *Aglais urticae* was the next most frequent species (almost 18% of locations). This is the only shelter type where three hibernating individuals of *Nymphalis polychloros* were found. In the natural caves, only a few hibernating individuals of *Inachis io* were found. This species was found in four out of 18 caves. *Inachis io* used more than one-third of 139 tunnels/galleries; *Aglais urticae* occurred there only marginally, with two records only. In unheated cellars, *Inachis io* was the typical species (more than one-half of 145 locations), while *Aglais urticae* was rarer there (15% of locations only) (see Table 1).

Inachis io was the most numerous species in all types of overwintering shelters. It prefers bunkers but also regularly occurrs in cellars. On the other hand, this species uses tunnels/galleries very sporadically; natural caves were the only other hibernation place recorded. Although the microclimatic conditions appeared to be very similar there, those shelters apparently are not as suitable for this species.

Aglais urticae has overwintering preferences similar to those of *Inachis io*. The primary difference in the results is that *A. urticae* is rarer in the study area than *I. io* and it also uses other, above ground, shelters for hibernation. *Aglais urticae* was recorded 11 times by itself, without the presence of *Inachis io* during the same visits, and 64 times together with *I. io*.

Nymphalis polychloros was found only on two separate occasions in military bunkers. At present, this species is the rarest species in the study area (Dvořák, pers.

Table 1. Summary of underground overwintering sites for Anglewing butterflies in the Czech Republic (W and SW Bohemia) - 10 years of records (winters of 1994/5-2003/4).

	# of sites	# of visits	Average	Maximum
Bunkers:				
	45	197		
Inachis io	37 (82%)	105 (53%)	6.2	28
Agalis urticae	8 (18%)	21 (11%)	2.7	11
Nymphalis polychloros	2 (4%)	2 (1%)	1.5	2
Caves:				
	18	109		
Inachis io	4 (22%)	5 (4.6%)	3.2	9
Tunnels/Galleries:				
	139	1336		
Inachis io	49 (35%)	232 (17%)	2.3	43
Aglais urticae	2 (1%)	2 (>1%)	1	1
Cellars:				
	145	623		
Inachis io	83 (57%)	271 (43.5%)	5.6	66
Aglais urticae	22 (15%)	52 (8.4%)	2.4	9
Total:				
	347	2265		
Inachis io	173 (50%)	613 (27%)	4.4	66
Aglais urticae	32 (9%)	75 (3%)	2.5	11
Nymphalis polychloros	2 (0.6%)	2 (0.1%)	1.5	2

Explanation of Table 1.

Bunkers – abandoned military concrete bunkers, mostly from World War 2 but some more recent.
Caves – natural karstic, pseudokarstic and fissure caves.
Tunnels/Galleries – entrances of abandoned mining tunnels and galleries.
Cellars – unheated cellars of houses, castles and monasteries.
Total – reported for all categories of overwintering shelters.
Numbers (#) of sites – total number of shelters surveyed with number of shelters (percentage in brackets) occupied by individual species (e.g. 45 bunkers were checked and 37 of them had *Inachis io*).
Numbers (#) of visits – total number of visits to any one type of shelter and number of positive checks (percentage in brackets) for each species (e.g. out of 197 checks of bunkers, 105 were positive for *Inachis. io*).
Average – the average number of butterflies per site that had hibernating individuals present.
Maximum – the maximum number of specimens of each species observed in a single overwintering site.

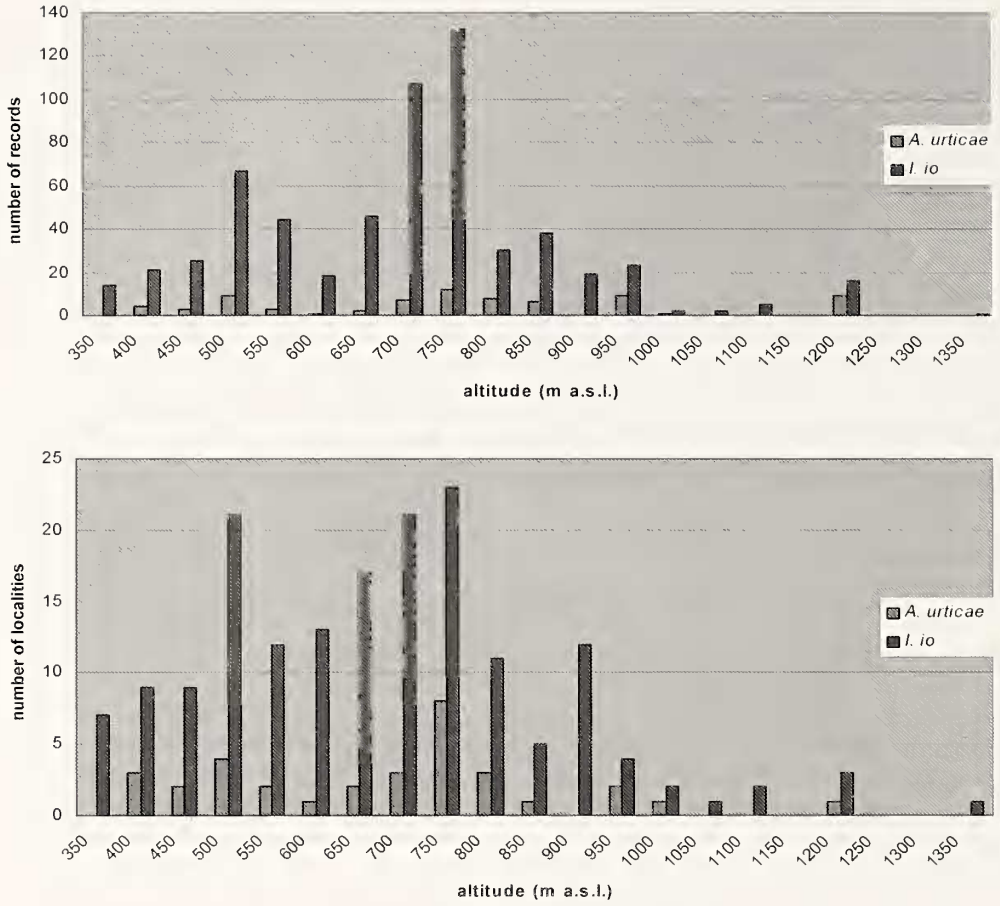

Figure 2. Comparison of abundance of *Inachis io* and *Aglais urticae* at different elevations.

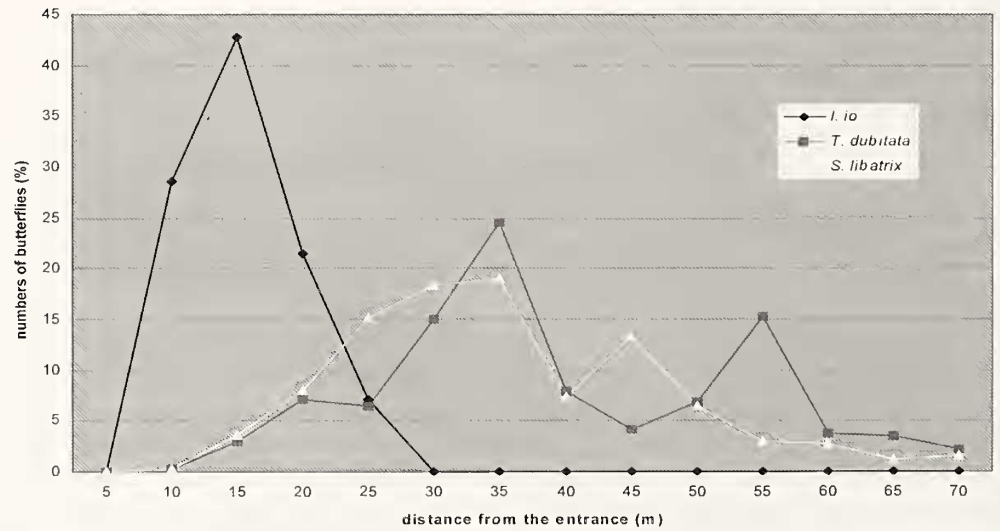

Figure 3. Comparison of abundance of *Inachis io* and two moths (*Scoliopteryx libatrix*, *Triphosa dubitata*) by distance from the entrance of one limestone gallery.

Table 2. Mass overwintering roosts (W and SW Bohemia) - maximum numbers found.

Locality:/Species:	Date	# individuals
Inachis io		
Klášter Teplá, cellars of a monastery	31.I.2003	66 ex.
Zhůří, cellars of a house, nr. Huťská Hora Mt.	18.X.1996	48 ex.
Okrouhlá, cellars of a fort	24.II.2003	47 ex.
Amálino Údolí, mining tunnel/gallery "Sněmovní"	23.XI.1995	43 ex.
Zhůří, cellars of a house, nr. Huťská Hora Mt.	13.XI.1997	41 ex.
Kašperk, cellars of a castle	5.XII.1996	38 ex.
Aglais urticae		
Záhvozdí, military bunker SE of the village	13.IX.1998	11 ex.
Záhvozdí, military bunker SE of the village	15.XI.1998	9 ex.
Klášter Teplá, cellars of a monastery	2.II.2004	9 ex.
Zhůří, cellars of a house, nr. Huťská Hora Mt.	7.IV.2004	9 ex.
Ostroh, cellars of a castle Seeberg	3.II.2004	7 ex.

observation). The closely related species *N. antiopa* is more common than *N. polychloros* in Bohemia. The fact that *N. antiopa* was never found in the underground shelters - although the rare *N. polychloros* was - indicates that the two species do differ in hibernation sites, and that *N. antiopa* may not use underground shelters at all.

Hypsometric distribution

Both *Aglais urticae* and *Inachis io* have a visible peak in number of overwintering records at an altitude 500 and 750 m a. s. l., judging from number of records and number of locations (Fig. 2). This result may be strongly influenced by the fact that the majority of shelters were in those altitudes, however it apparent the occurrence curve is the similar for both species. Fig. 2 corroborates the conclusion that both species have similar requirements for underground winter shelter, and probably the only difference is that *I. io* is the more common species of the two.

Numbers of individuals

A large number of individuals were found hibernating together in only some underground shelters. In several locations, congregations were found repeatedly for several consecutive years. For some locations the dates of visits are listed, together with the greatest numbers of wintering butterflies (Table 2). From those data it is also evident that (i) both species prefer cellars and/or bunkers and (ii) *A. urticae* is less frequently found in underground shelters than is *Inachis io*.

Temperature

The air temperature in the shelter is one of the factors significantly influencing the overwintering of Nymphalini (Pullin & Bale, 1989a, b). *Inachis io* was found during winter in shelters at temperatures between −1.1 and +14.6°C. It is interesting to note that *I. io* was found hibernating relatively close to the entrance of the gallery, in contrast to the winter diapausing moths *Scoliopteryx libatrix* and *Triphosa dubitata*, whose occurrence peaked about 15 m in from the entrance (Fig. 3).

Differences between years

We found very strong differences between the occurrence of *Inachis io* and *Aglais urticae* in different years (F_{10}, 1330=14.95, p<<0.0001 and F_{10}, 1330=85.4, p<<0.0001, respectively). For the analyses, the most reasonable was entering year as the factor variable. For *I. io*, when year was treated as a continuous variable, it explained 0.7% of the variability; when year was treated as a categorical variable, it explained 12.9% of the variability. For *A. urticae*, when year was treated as a continuous variable, it explained 30.8% of the variability; when year was treated as a categorical variable, it explained 49.5% of the variability. This means that between-year variation is strongly irregular, with the pattern much more complicated in *I. io* than in

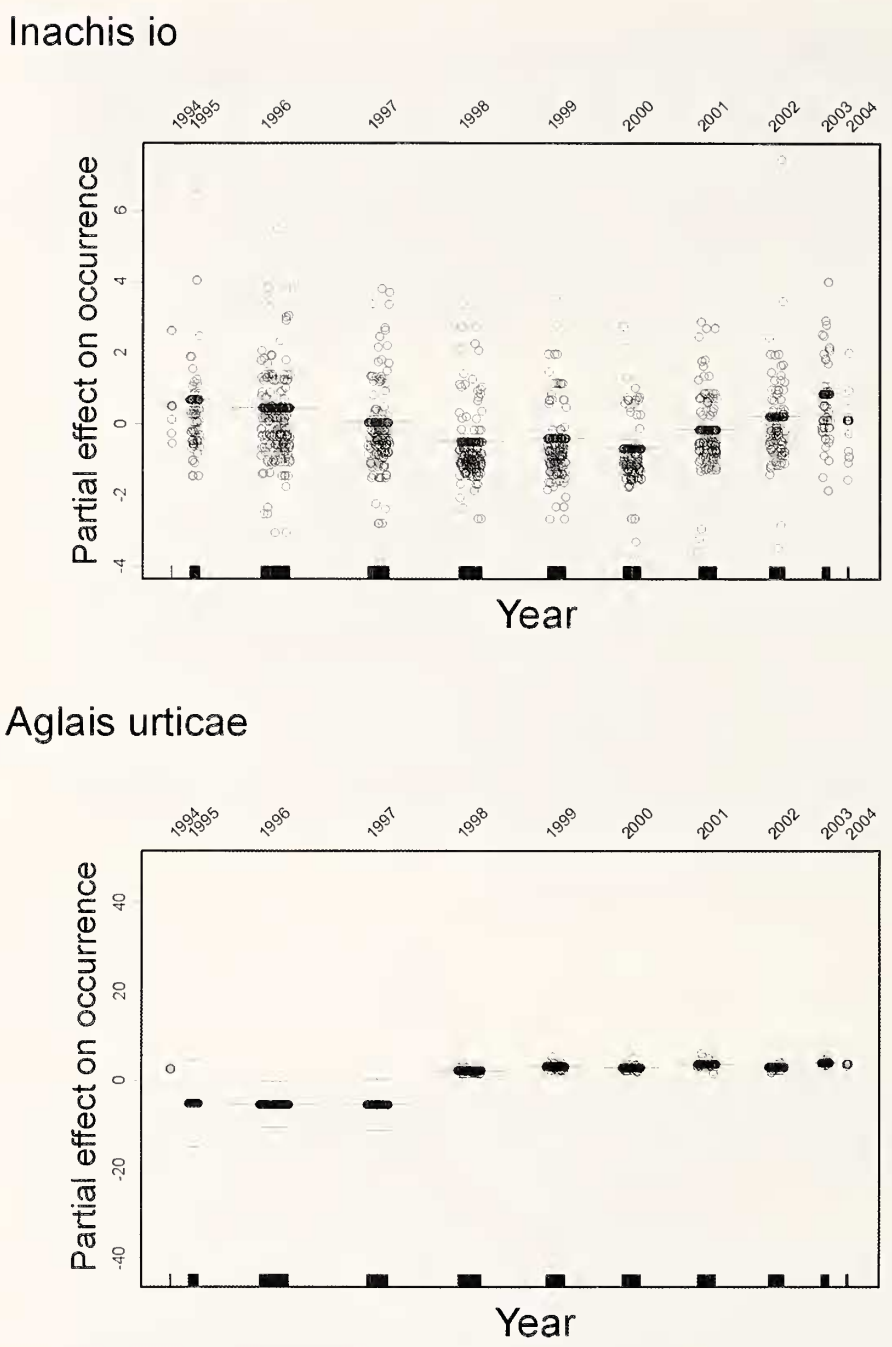

Figure 4. Effect of between-year variation on occurrence (in natural logarithms) of *Inachis io* and *Aglais urticae* in underground shelters, after filtering out the effects of place and site.

A. urticae (Fig. 4). There was also a strong correlation between occurrence of *I. io* and occurrence of *A. urticae* (F_1, 1339=11.18, $p < 0.001$).

Discussion

It appears that *Vanessa atalanta* is not an obligatory hibernator in the Czech Republic (Belicek, pers. observations), and hence this species was not observed hibernating during this study. According to Higgins and Riley (1978) and others, these butterflies either migrate south or die during autumn in Europe. One of the few published records of overwintering individuals of *V. atalanta* was from a military bunker NW of Krefeld

(Hülser Bergs foothills, western Germany). One individual was found hibernating there on 19.I.1997, together with individuals of *Aglais urticae* and *Inachis io*, by Bäumler in Hensle (2000). Another case of hibernating *V. atalanta* is from a graphite gallery/ mining tunnel in the study area, but this is observation is more than 30 years old (Majer *in* Dvořák 2000).

From the observations made over a 10-year period, it seems likely that *Nymphalis polychloros* only very rarely hibernate in underground shelters. Dvořák found one individual of this species in a natural cave of the Bohemian Karst (central Bohemia) that was in summer aestival diapause (Dvořák, 2002). The two records reported here from abandoned military bunkers in the Bohemian Forest foothills (SW Bohemia) are the first documented examples from the study area.

It is often stated that *Aglais urticae* partly immigrates into the region from the south, and that the immigration is supplemented by local hibernation. Underground shelters probably represent only a part of the spectrum of shelters used. The species is rarely found in natural caves of central Europe (*cf.* Kowalski, 1955; Bronner, 1987). *A. urticae* was not found in any cave visited in the study area. There are very few published observations from other underground shelters. Several specimens were observed hibernating in Germany, e.g. in hollows of highway bridges in Oberberg region (Herhaus & Karthaus, 1996) or in bunkers (Hensle, 2000). As far as known to us, no mass winter roosts of this species have been reported in the literature. The observed congregations of up to 11 individuals reported here are noteworthy.

On the other hand, records of hibernating *Inachis io* from different underground shelters are relatively numerous; being reported also from caves (e.g. Kowalski, 1955; Košel, 1984; Dvořák, 2002) and other underground shelters. The highest number reported was 46 individuals in one bunker (Hensle, 2000), and up to 60 individuals in a cavity of a highway bridge (Herhaus & Karthaus, 1996). The 66 individuals found in a cellar of a monastery was the highest number recorded in this survey.

Conclusions

The three nymphalid species *Inachis io*, *Aglais urticae*, and *Nymphalis polychloros* were observed using underground shelters in SW and W Bohemia as their overwintering sites.

Inachis io was the most common species in underground shelters, while *Aglais urticae* was less numerous.

Unheated cellars and abandoned military bunkers were the predominant wintering sites, followed by entrances to mining tunnels/galleries. A few records of *I. io* were from natural caves.

Inachis io was commonly observed in mass aggregations in winter shelters (up to 66 individuals), while groups of *Aglais urticae* were found only occasionally and in smaller numbers.

Only three specimens of *Nymphalis polychloros* were found individually in abandoned military bunkers.

In terms of butterfly conservation and protection of natural resources, it is apparent that wintering sites such as abandoned military bunkers, entrances to mining tunnels/galleries, caves, etc. are important for the winter survival of these butterflies. Consequently, well known wintering sites should be protected during the winter from undue disturbance.

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APPENDIX

1. *Aglais urticae*, in cellars of a monastery in Teplá, W Bohemia, February 2004, photo J. Bartoš.
2. *Inachis io*, in an abandoned military bunker near Opolenec, SW Bohemia, January 2004, photo M. Rudlová.
3. *Nymphalis polychloros*, in an abandoned military bunker near Tuškov, SW Bohemia, January 2004, photo V. Vlk.
4. A building of a former hotel in Bečov nad Teplou, W Bohemia, February 2003, hibernation place of *Inachis io* and *Nymphalis polychloros*, photo V. Vlk.
5. The Entrance to an underground gallery near Kašperské Hory, SW Bohemia, May 2004, in cellars were found hibernating *Inachis io* and *Aglais urticae*, photo J. Bartoš.
6. An abandoned military bunker near Tuškov, SW Bohemia, May 2004, hibernation place of *Inachis io*, photo V. Vlk.